J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 981–992

Two-Dimensional Electrophoresis of Proteins in Tumours of the Lung

By *A. T. Endler, D. S. Young*

Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA
and
Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA, USA

L. E. Wold

Department of Pathology, Mayo Clinic, Rochester, MN, USA

M. M. Lieber

Department of Urology, Mayo Clinic, Rochester, MN, USA and

Rose M. Currie

Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA

(Received April 14/July 3, 1986)

Summary: The proteins of solid lung tumours (15 adenocarcinomas and 10 squamous cell carcinomas) were examined by high resolution two-dimensional electrophoresis (2-DE) and compared with the proteins of adjacent lung tissue which demonstrated no histological evidence of malignant transformation, and with the proteins of other malignant tumours and normal tissues. To investigate tumour cell-specific protein synthesis, we isolated malignant and normal cells enzymatically with collagenase, elastase, and DNase. Tissue and tumour cells were enriched in an additional step on a Percoll gradient. The 2-DE gel patterns derived from entire tissue and enriched tissue cell preparations were compared.

No specific differences were found between the 2-DE protein patterns from adenocarcinomas and squamous cell carcinomas of the lung, but three proteins identified on the 2-DE gels appeared to be tumour-associated. Spot A is present in non-neoplastic and neoplastic epithelial tissues. Spot B is pronounced in 2-DE gels of sarcomas, but is also present in preparations of other malignant tissues. Spot C is present in all malignant cell preparations. These three spots were also demonstrated in 2-DE protein patterns from tissue cultures of malignant cell lines. Spot B and spot C were also present in some normal tissues.

Zweidimensionale Elektrophorese von Proteinen in Lungentumoren

Zusammenfassung: Solide Lungentumoren (15 Adenocarcinome und 10 squamöse Zellcarcinome wurden mit der hochauflösenden zweidimensionalen Elektrophorese (2-DE) untersucht. Diese Tumoren wurden mit dem angrenzenden Lungengewebe, das keine histologischen Hinweise auf maligne Transformationen zeigte und mit anderen malignen Tumoren und normalen Geweben verglichen. Um die tumorspezifische Proteinsynthese zu studieren, isolierten wir maligne und normale Zellen aus soliden Tumoren und Geweben enzymatisch mit Kollagenase, Elastase und DNase. In einem zusätzlichen Schritt wurden Gewebe- und Tumorzellen auf einem Percoll-Gradienten angereichert. 2-DE-Gele vom gesamten Gewebe und angereicherten Gewebezellpräparationen wurden verglichen.

Die Adenocarcinome und squamösen Zellcarcinome der Lunge wiesen in der 2-DE keine spezifischen Unterschiede auf. Drei tumor-assoziierte Proteine konnten auf den Gelen identifiziert werden. Fleck A befindet sich in nicht-neoplastischen und neoplastischen epithelialen Geweben. Fleck B kommt insbesondere in 2-DE-Gelen von Sarkomen, aber auch in anderen malignen Geweben vor. Fleck C zeigt sich in 2-DE-Gelen aller maligner Zellpräparationen. Diese drei Proteinflecke ließen sich auch in 2-DE-Gelen von Zellkulturen maligner Zellinien nachweisen. Fleck B und C fanden sich auch in einigen normalen Geweben.

Introduction

Cancer cells are pathologically transformed cells with aberrant differentiation and generally increased proliferative activity. These features occur in connection with genetic changes which cause variations of the cellular protein composition of the cell (1). In 1953, *Foley* first provided evidence of the appearance of a new protein (neoantigen) in chemically induced malignant tumours in an animal model (2). Today, we recognize that malignant cells have the ability to form new proteins and that some proteins which are normally present may be modified, augmented, diminished or even eliminated.

High resolution two-dimensional electrophoresis (2-DE), as developed by *O'Farrell* (3), achieves better resolution of proteins in biological material (e. g. serum, urine, cerebrospinal fluid, cells) than any other single procedure (4). Ten thousand different polypeptides can be revealed in a single human cell (5). 2-DE may also be used to search for genetic variabilities (4–7) and to search for and investigate the modifications of protein patterns in transformed cells (8).

In this study we focused on pulmonary malignancies, because they are the most common cancers in men, and have emerged as a common cancer in women also (9). We investigated whether a relationship exists between the histology and the protein composition of lung tumour tissues, as well as other non-neoplastic and neoplastic tissues.

The protein composition of enriched cell preparations from malignant lung tumours and from adjacent normal tissues were examined by 2-DE. Other non-pulmonary normal tissues and malignant tumours were also examined. In addition to examining solid tissues, as reported by others (10–13), we also investigated the differences between enriched cell preparations and whole tissue preparations by 2-DE. We also compared tissues from patients with in vitro cell lines of malignant tumours.

Materials and Methods

Specimen acquisition

Tumour tissue was obtained from patients undergoing surgical resection of their malignancies at the Mayo Clinic. Frozen section examination was immediately performed. Part of the homogenous tumour tissue was isolated, packed in a plastic bag, sealed and sent immediately to the laboratory by pneumatic tube. In some instances adjacent tissue which was histologically normal, showing no evidence of malignant transformation, was also resected and processed in the same way. The time interval between surgical removal of the tissue and beginning of tissue processing for 2-DE in the laboratory did not exceed 45 minutes. The investigated malignant tissues and normal tissues are listed in table 1.

Enzymatic digestion of tissue

Approximately 400 mg of wet tissue was rinsed in phosphate-buffered saline pH 7.2 to remove blood cells. The tissue was then minced into small pieces with scissors and suspended in 10.0 ml Minimal Essential Medium (GIBCO, Grand Island, NY) containing 1.00 g/l collagenase, 2.00 g/l elastase, 0.01 g/l DNase (all enzymes from Worthington Diagnostic Systems Inc., Freehold, NJ), 20 g/l bovine serum albumin (Sigma Chemical Co., St. Louis, MO) with 5 units penicillin and 5 µg streptomycin (GIBCO, Grand Island, NY) per ml. This suspension was incubated for 1 hour at 37 °C. Undigested tissue clumps were removed by passing cells through a nylon mesh. Cells were washed twice in enzyme-free medium with bovine serum albumin and antibiotics and subsequently centrifuged at 400 *g* for 10 minutes at 4 °C.

Enrichment of cells from normal and malignant tissue

We separated tumour and normal tissue cells from contaminating blood cells and debris on a discontinuous gradient of 45% of a colloidal suspension of silica particles coated with polyvinylpyridine (Percoll) (Pharmacia, Piscataway, NJ) with centrifugation for 15 minutes at 800 *g* at 4 °C. Cells were recovered from the interface and washed three times with centrifugation at 400 *g* for 10 minutes at 4 °C in phosphate-buffered saline.

Viability of cells was determined by trypan blue exclusion. Only preparations with a viability over 90% were used for further processing. Cytological preparations were done on a Cytospin centrifuge (Shandon Southern Instruments, Inc., Sewickley, PA) and stained with *Wright* stain or with haemotoxylin-eosin.

In vitro cell lines (tab. 1)

Continuous tumour cell lines were propagated as monolayer cultures in 75 cm^2 tissue flasks (Falcon, Subdivision of Becton, Dickinson and Co., Oxnard, CA). Cells were grown in a solution containing 9 parts *Dulbecco*'s Modified Eagle Medium (GIBCO, Grand Island, NY) and 1 part calf serum. Cells were passaged gently by exposure to a dilute solution of trypsin (GIBCO, Grand Island, NY) and EDTA. The cell cultures were maintained at 37 °C in a 95% air and 5% CO_2 environment in a tissue culture incubator (Wedco, Inc., St. Augustine, FL). Cells were harvested for analytical studies during log phase growth. They were scraped from the monolayers using a "rubber policeman", and were not exposed to trypsin. Then they were washed three times by centrifugation at 400 *g* for 10 minutes at 4 °C in phosphate-buffered saline. Viability was checked with a trypan blue exclusion test and was always above 90%.

Tab. 1. Analysed tissues and cell lines.

Type	Quantity
Lung adenocarcinoma	15
Lung squamous cell carcinoma	10
Lung other tumours	3 (2 large cell carcinoma, 1 leiomyosarcoma)
Lung normal	16
Metastasis from lung tumours in other organs	2 (squamous cell carcinoma in lymph nodes)
Metastasis in the lung from non lung primaries	6 (4 sarcoma, 1 colon adenocarcinoma, 1 transitional carcinoma from bladder)
Normal organs	15 (2 liver, ileum [2 glandular mucosa and 1 muscularis], colon [2 glandular mucosa and 2 muscularis], 1 testis, 2 skin*, 1 parotid gland, 2 buffy coat)
Other malignant tumours	13 (3 renal cell carcinoma, 1 hepatoma, 2 adenocarcinoma of the stomach, 2 adenocarcinoma of the ovaries, 4 adenocarcinoma of the breast, 1 transitional cell carcinoma of the bladder)
Metastasis from non-pulmonary tumours in other organs than lung	11 (1 in hip from breast adenocarcinoma, 1 in lymph node from melanoma, 2 in liver from colon adenocarcinoma, 4 in lymph node from breast adenocarcinoma, 1 in serosa from endometrial carcinoma, 1 in m. scalenus from dysgerminoma, 1 in peritoneum from liposarcoma)
Cell lines	6: 1 CALU-3**) adenocarcinoma of the lung 1 SK-MES-1**) squamous cell carcinoma of the lung 1 A-427**) lung carcinoma 1 A-1663**) transitional cell carcinoma from the urinary bladder 1 SW-480**) adenocarcinoma of the colon 1 MEWO***) melanoma

*) Entire tissue preparation
**) From ATCC, Rockville, MD
***) From Dr. *F. Fogh*, Memorial Sloan-Kettering Cancer Institute, New York, NY

Cell homogenization and preparation for isoelectric focusing

A modification of the method described by *Tracy* et al. (10) was used for homogenization of cells and dissociation of their proteins and peptides. Cells (13×10^6) were mixed with 100 µl homogenization buffer (per litre: 8 mol of urea and 50 ml 2-mercaptoethanol). Proteolysis was inhibited by addition of 5 µl of a PMSF-pepstatin solution (25 mg phenylmethylsulphonyl fluoride and 1 mg pepstatin disolved in 1.5 ml ethanol) and 5 µl benzamidine solution (16 mg benzamidine in 1 ml distilled water) to 13×10^6 cells.

Cell preparations were either stored at −70 °C or were subjected to further processing immediately, because freezing at later stages resulted in inconsistent 2-DE patterns.

Sodium dodecylsulphate solution (10 µl; 100 g/l SDS) was added to 100 µl of cell homogenate and the sample was homogenized in a microsized glass grinder. At this point 33 µl of dissociation buffer (containing 8 mol of urea, 80 ml of surfactant Nonidet P-40, and 50 g of ampholyte [3 parts pH 3.5−10 and 1 part pH 5−7] [LKB Instruments, Inc., Gaithesburg, MD] per liter) was added. Samples were centrifuged on a microfuge (Beckman Instruments, Irvine, CA) for 5 minutes. Twenty microlitres were carefully aspirated from the solution in the middle of the tube without disturbing the bottom of the tube and the floating lipid layer. Duplicate 10 µl samples (equivalent to 6×10^5 cells) were immediately applied to isoelectric focusing gels and electrophoresed.

Homogenization and preparation for isoelectric focusing of entire tissue

In several cases some tissue was cut into a small block and immediately frozen at −70 °C. Tissue was sliced from the block and was homogenized and prepared in a way similar to that used for the enriched cell specimens, following a previously described procedure (10).

Two-dimensional electrophoresis (2-DE)

We used a modification of *Anderson* and *Anderson*'s ISO-DALT technique (14) to separate proteins, as described elsewhere (15). In the first dimension, isoelectric focusing gels contained 35 g/l acrylamide and 19 g/l of pH 3.5−10 and 5 g/l of pH 5−7 ampholytes.

The second or DALT dimension was run on a SDS-polyacrylamide gradient from 10% to 23%. Proteins were made visible with the silver stain, using the modification of *Oakley* et al. (15, 16, 17). All gels were photographed with the acidic side to the left and the largest molecules at the top.

The pH gradient of isoelectric focusing gels was determined by cutting a pair from each batch into 15 equal parts. Each part was put into 0.5 ml degassed distilled water. After incubation for at least 1 hour on a shaker the pH was measured. Molecular weight markers were run simultaneously on the SDS-polyacrylamide gel for determination of molecular weight.

Results

Using routine histological techniques, we confirmed that the tumour tissues used in the present study consisted predominantly of malignant cells (fig. 1). We also investigated tissues of normal appearance, taken from a variety of sites adjacent to the malignancy without histological signs of neoplasia. When tumour cell lines were examined these contained only one pure cell population.

Enriched cell preparations

We isolated cells from solid malignant tumours and from normal tissues. Non-fibrous tissues were subjected to enzymatic digestion to produce enough cells for 2-DE analysis. Tissues which could not be digested with the enzyme mixture were analysed as

whole tissue. Since elastin and collagen are the major structural components of lung tissue, we used the specific enzymes collagenase and elastase to digest the intercellular matrix, avoiding unspecific proteases like trypsin or chymotrypsin which might also digest cell membrane proteins. In previous experiments we found that the addition of DNase improved the yield of cells. An effective non-toxic enzyme concentration was determined in previous trials, checking cell viability with a trypan blue exclusion test.

The majority of contaminating blood cells and debris can be removed on a discontinuous 45% silica gel gradient. We found that this concentration of Percoll produced optimal results using a modification of the method of *Lindena* et al. who separated cells from whole blood (17). Blood cell contamination was checked in cytocentrifuge preparations and did not exceed 5% (fig. 1 b, d, e).

To diminish effects of proteolysis occurring in vivo and during cell preparation, only preparations with a cellular viability of 90% or greater were subjected to analysis. The protease inhibitors pepstatin, phenylmethylsulphonyl fluoride, and benzamidine were added to impede destruction of cellular proteins during storage, homogenization, and dissociation of the specimen.

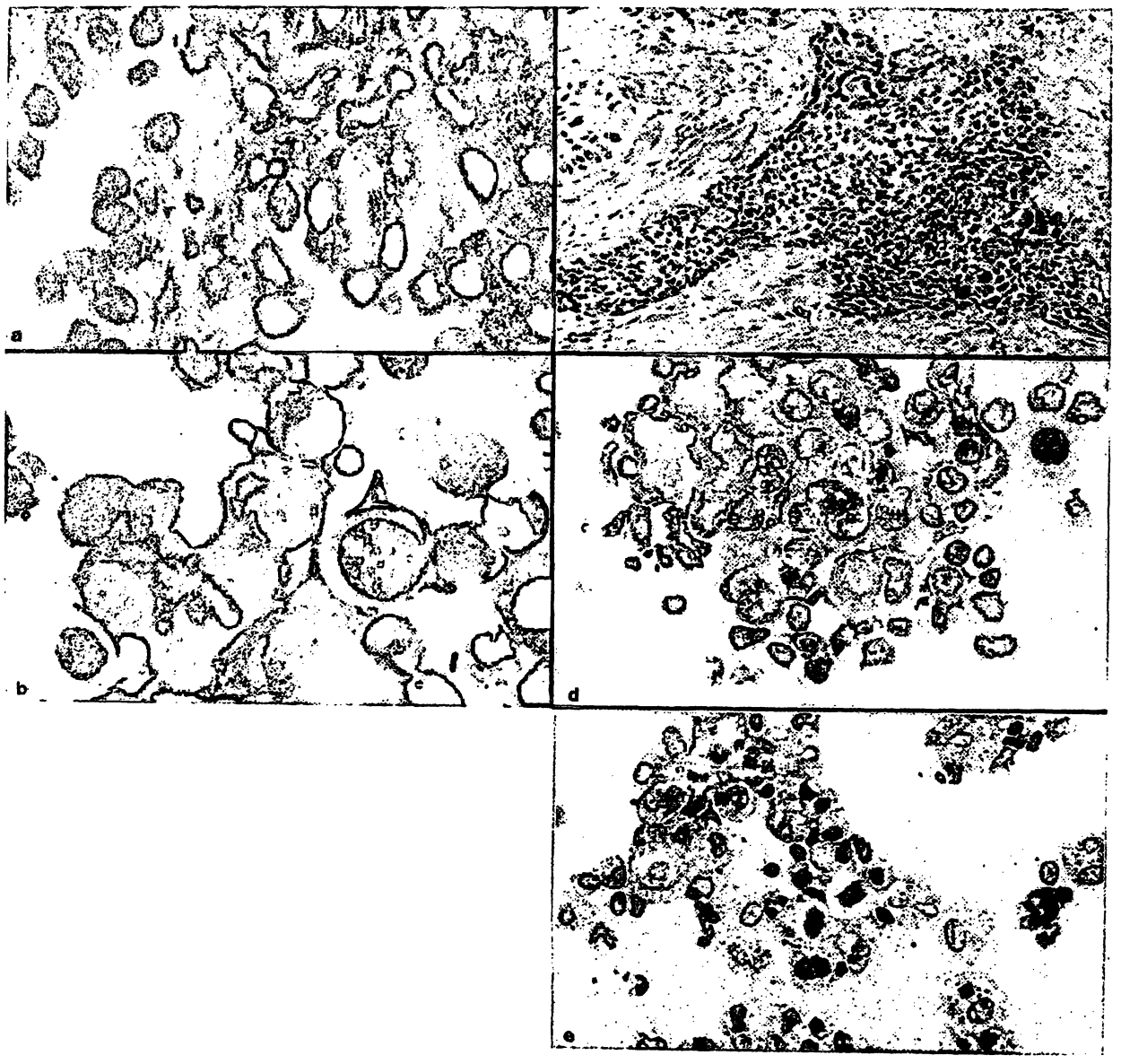

Fig. 1. Histological pictures of
a) adenocarcinoma of the lung (× 1000) and
b) its enriched cell preparation (cytocentrifuge preparation) (× 1000);
c) squamous cell carcinoma of the lung (× 160) and
d) its enriched cell preparation (cytocentrifuge preparation) (× 400);
e) enriched cell preparation (cytocentrifuge preparation) (× 400) of normal pulmonary parenchyma showing inflammatory cells and alveolar lining cells.

It is difficult to analyse normal lung tissue, because of its high content of interstitial fluid. The abundance of plasma proteins in the specimen contributes to poor resolution and makes it difficult to identify proteins restricted to the lung itself. Fibrous tissues like tendon or normal skin or fibrous tumours do not yield a sufficient quantity of cells for 2-DE, even with enzymatic digestion. Therefore we analysed these tissues in their entirety on 2-DE.

As an example for the influence of plasma proteins and other interstitial proteins and peptides on 2-DE gel patterns we prepared the same tissue of an ovarian adenocarcinoma by the two described methods and compared them (fig. 2). In the total tissue preparation, plasma proteins can be recognized, especially albumin, which is superimposed on other proteins. In the preparation of the entire tissue more low molecular weight proteins and peptides can be recognized than in the enriched cell preparation which might indicate increased proteolytic activity. Entire normal tissues do not show as many low molecular weight proteins and peptides. Some spots are more pronounced or are only visible in the 2-DE picture of the enriched cell preparation, thus indicating their cellular origin. Proteins enhanced or only present in 2-DE gels of the tissue preparations are probably interstitial fluid proteins, plasma proteins or products of necrosis, or proteolysis.

To investigate the proteolytic activity of collagenase, elastase, and DNase we incubated the enzymes with normal human serum under the same conditions as described previously for the isolation of tissue cells. The 2-DE gels of the original serum and incubated serum showed no obvious differences. Therefore we conclude that components of the enzyme mix used in the isolation of cells are not responsible for differences in the gel pattern between enriched cell preparation and whole tissue.

Cells from tissue cultures are not contaminated by serum proteins since they are removed by washing cells in a non-protein containing medium.

Enriched cell preparations of homogeneous tumour tissue provide a homogeneous cell preparation, while normal tissue, especially from the lung, usually contains a varied cell population (fig. 1 e). The 2-DE separations are reproducible within one organ and within one tumour within one person. The same organ (e. g. lung) from different people produces some differences in 2-DE gels (fig. 3 and 4).

In two instances we analysed normal lung tissue, tissue from a squamous cell carcinoma of the lung, and tissue from a corresponding peripheral lymph node metastasis in the same patients. Many features of the malignant tumour cells, which were not present in the 2-DE gel of the corresponding normal lung

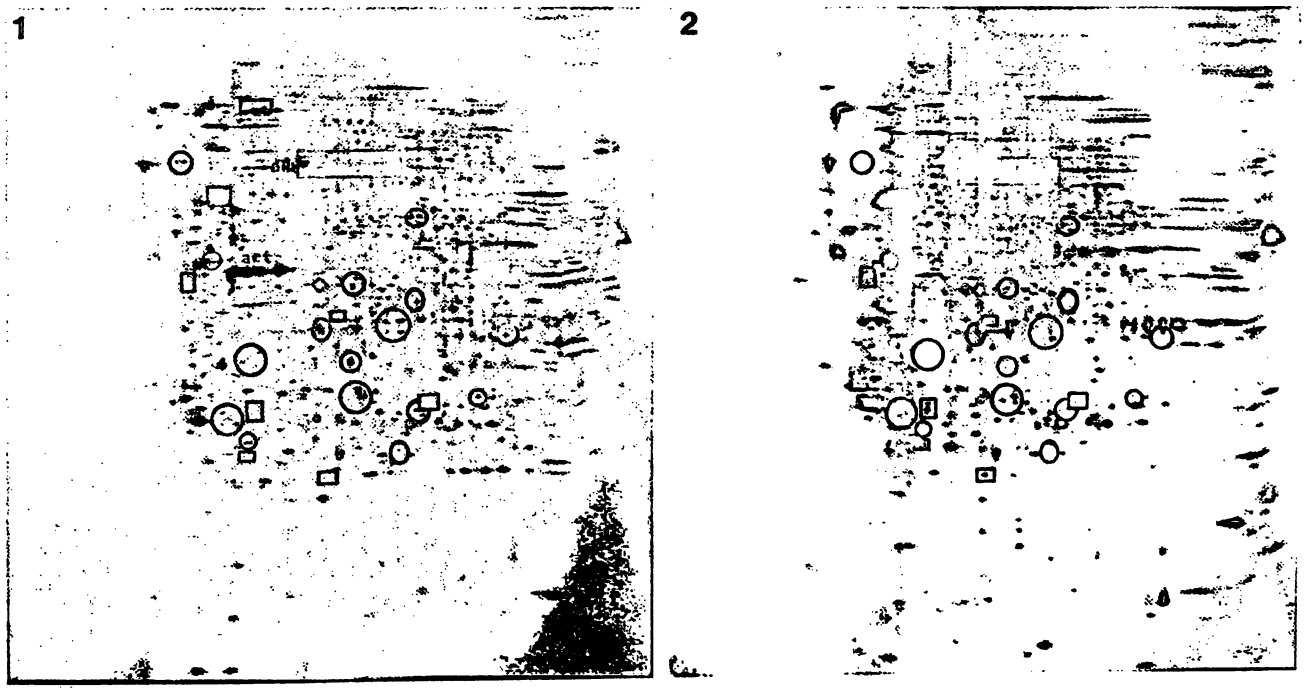

Fig. 2. Comparison of 2-DE gel patterns from an adenocarcinoma of the ovary. Probes were taken from the same specimen at the same site:
(1) enriched cell preparation,
(2) whole tissue preparation.
Spots in circled areas are augmented or only seen in the enriched cell preparation, while spots in areas denoted by a rectangle are augmented or only seen in whole tissue preparation (alb = albumin, act = actin).

tissue, were present in the malignant cell preparation of the metastasis including the malignancy-associated proteins (fig. 4).

Comparing 2-DE gels from normal lung tissue, squamous cell carcinoma of the lung, and adenocarcinoma of the lung we found three spots commonly present in malignant lung tumours but much less common in other tissues (figs. 3, 4, 5, 6). The frequency of these three spots is listed in table 2. The spots have the following characteristics:

The protein of spot A, which is associated with cancer and epithelial tissue (figs. 3, 4, 5), has an approximate molecular weight of 27 × 10^3 daltons and an approximate isoelectric point at pH 5.35 in our system. It is present in all pulmonary carcinomas including adenocarcinomas, squamous cell carcinomas, large cell carcinomas, and their metastases. Spot A was also found in most of non-pulmonary neoplastic tissues, except sarcoma tissues. Only one sarcoma, an epitheloid sarcoma, contained spot A as demonstrated by 2-DE. Six out of twelve normal lung tissues contained spot A. Normal skin, mucosa of the ilium and colon, and parotid showed this protein in 2-DE, while liver, testis, buffy coat, and muscularis of the intestine (except in a single case) had not detectable spot A.

The protein of spot B, which is associated with malignancies (figs. 3, 4, 6), has an approximate molecular weight of 42 × 10^3 daltons and is located approximately at pH 6.25 on the 2-DE gels. This protein was present in two thirds of the investigated adenocarcinoma of the lung and in half of the squamous cell carcinoma of the lung. Out of 24 other carcinomas from different sites and types only six demonstrated spot B in 2-DE gels, while all investigated sarcomas contained large amounts of this protein. In the 2-DE gels of normal tissues, spot B was found in 2 out of 16 lung tissues, in one liver preparation, in the testis and in the muscularis of the colon and the ilium. This protein was also present in one preparation of the mucosa of the colon. It was also present in cell lines, except MEWO and A-1663.

The protein of spot C, which is also associated with malignancies (figs. 3, 4), has an approximate molecular weight of 51 × 10^3 daltons and a pI ranging from approximately pH 6.4 to pH 6.6 in our system. This protein appears in all 2-DE gels of carcinomas of the lung (except one adenocarcinoma) and in all other analysed carcinomas. Spot C was also present in 4 out of 6 sarcomas and in all investigated tissue cultures of malignant tumours. Spot C occurs with the same frequency in normal lung tissue as spot B, i.e. 2 out of 16. It was also found in preparations of the liver and of the parotid gland. All specimens from the mucosa of the intestine and one preparation of the muscularis of the colon contained spot C.

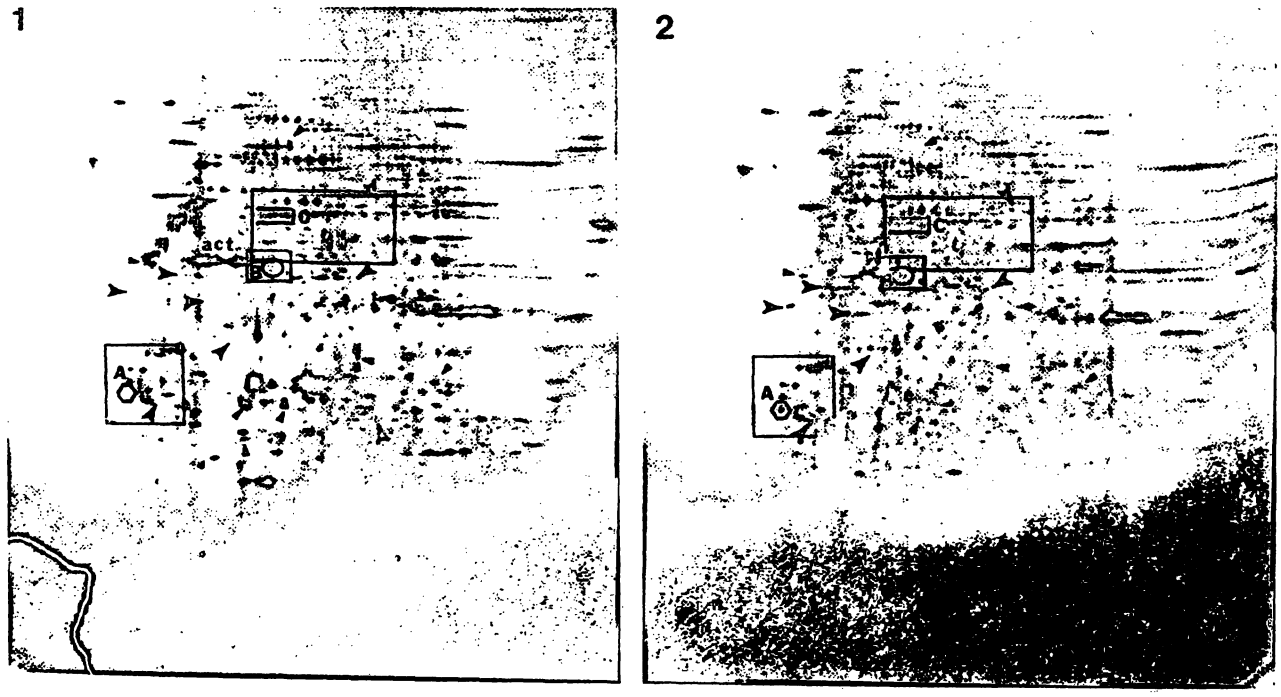

Fig. 3. Comparison of 2-DE gels produced from enriched cell preparations from the lung:
(1) normal lung tissue,
(2) tumour tissue from an adenacarcinoma of the lung from the same patient.
Spots marked with small arrows are mainly present in normal tissue and spots predominant in malignant tissue are marked with large arrows (act = actin). The areas with the three tumour-related proteins are marked as (A), (B), and (C) within rectangles.

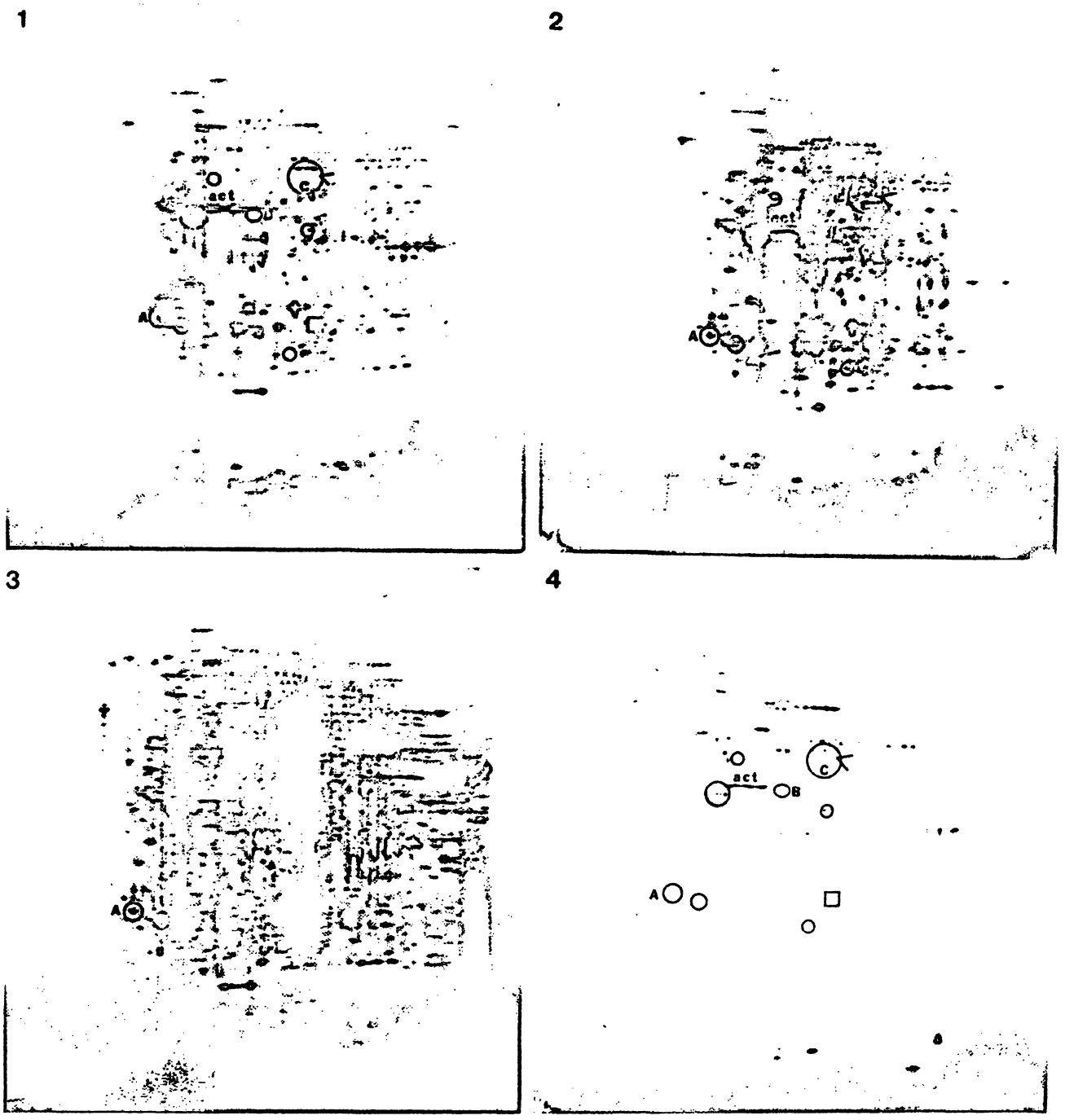

Fig. 4. Enriched cell preparation from a patient with squamous cell carcinoma of the lung and a peripheral metastasis:
(1) normal lung tissue,
(2) and (3) squamous cell carcinoma from different sites within the same tumour,
(4) lymph node metastasis from the primary tumour (only 3×10^5 cells were analysed in this 2-DE gel because only a small amount of tissue was available for processing).
Spots present in larger amount or only present in malignant tissue preparation, but not in normal tissue preparation, are circled. Spots in larger amount or only present in normal tissue, but not in malignant tissue are marked with rectangles (act = actin). The three tumour-related spot areas are marked (A), (B), and (C).

Discussion

In this study we focused of squamous cell carcinoma and adenocarcinoma on the lung. The main problem in studying pulmonary malignancies is the isolation and preparation of suitable control material. Therefore we compared lung tumour tissues with adjacent lung tissue which was normal, not showing any evidence of malignant transformation on histologic inspection. Nevertheless, tumour and proteins might have spread into these adjacent tissues without being detected histologically.

As the character of the original cell type which is transformed into a malignant cell and which comprises the malignant tumour is not always clearly defined, and as normal lung tissue is a mixture of several cell types (e.g. mesenchymal cells, epithelial cells, alveolar cells, brush border cells), we investigated additional normal tissues from other organs possessing cells with similar structure and functions (tab. 1). Other malignant tumours were investigated to determine whether specific proteins are present only in lung cancer tissues and/or also in other malignant tissues from different locations and of different origins. The reasons for investigating tissue cultures are firstly the uniform character of these cells, secondly the possible enhancement of malignant marker proteins, and thirdly their ready availability for further investigation. Several previous searches for cancer specific proteins have used tissue cultures as starting material (18–20).

Isoelectric focusing, the first dimension, is performed in polyacrylamide which limits the amount of sample to be analysed. Not all proteins and peptides loaded on the isoelectric focusing gel enter completely into the polyacrylamide, molecular weight being one of the main limiting factors. Additionally, the lowest detection limit of a given protein has been reported as 5 ng on 2-DE gels visualized with silver stain (15). Purification and concentration of the investigated material improves the recognition of specific proteins and peptides within a given protein mixture. Before analysing by 2-DE, preliminary preparation of a pro-

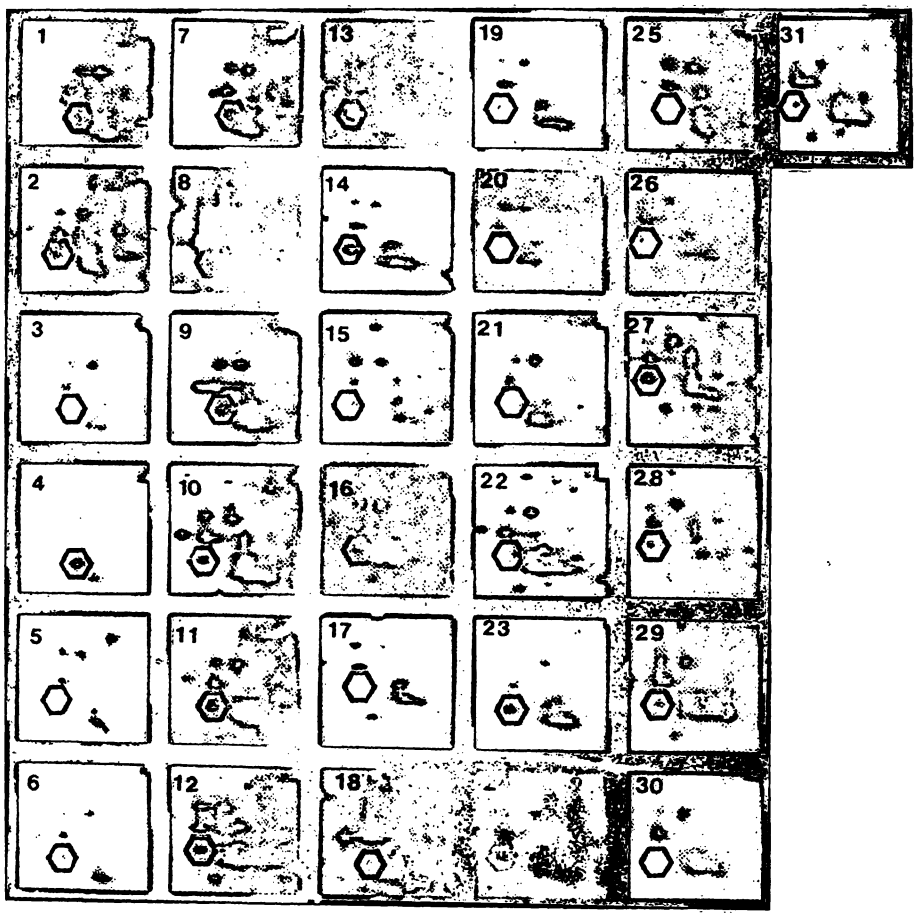

Fig. 5. 2-DE gels area of spot A as marked in figure 3 from the following tissues:

1–3	normal lung,
4	skin squamous epithelium,
5	buffy coat,
6	smooth muscle from colon,
7	mucosa from ileum,
8	liver,
9	parotid gland,
10–12	adenocarcinoma of the lung,
13–15	squamous cell carcinoma of the lung,
16	large cell carcinoma in the lung,
17	leiomyosarcoma in the lung,
18	metastasis of synovial sarcoma in the lung,
19	metastasis of epitheloid sarcoma in the lung,
20	adenocarcinoma of the stomach,
21	lymphoma in the testis,
22	renal cell carcinoma,
23	transitional cell carcinoma in the bladder,
24	adenocarcinoma of the breast,
25	liposarcoma, and the cell lines
26	CALU 3,
27	A-427,
28	SK-MES-1,
29	A-1663,
30	MEWO.

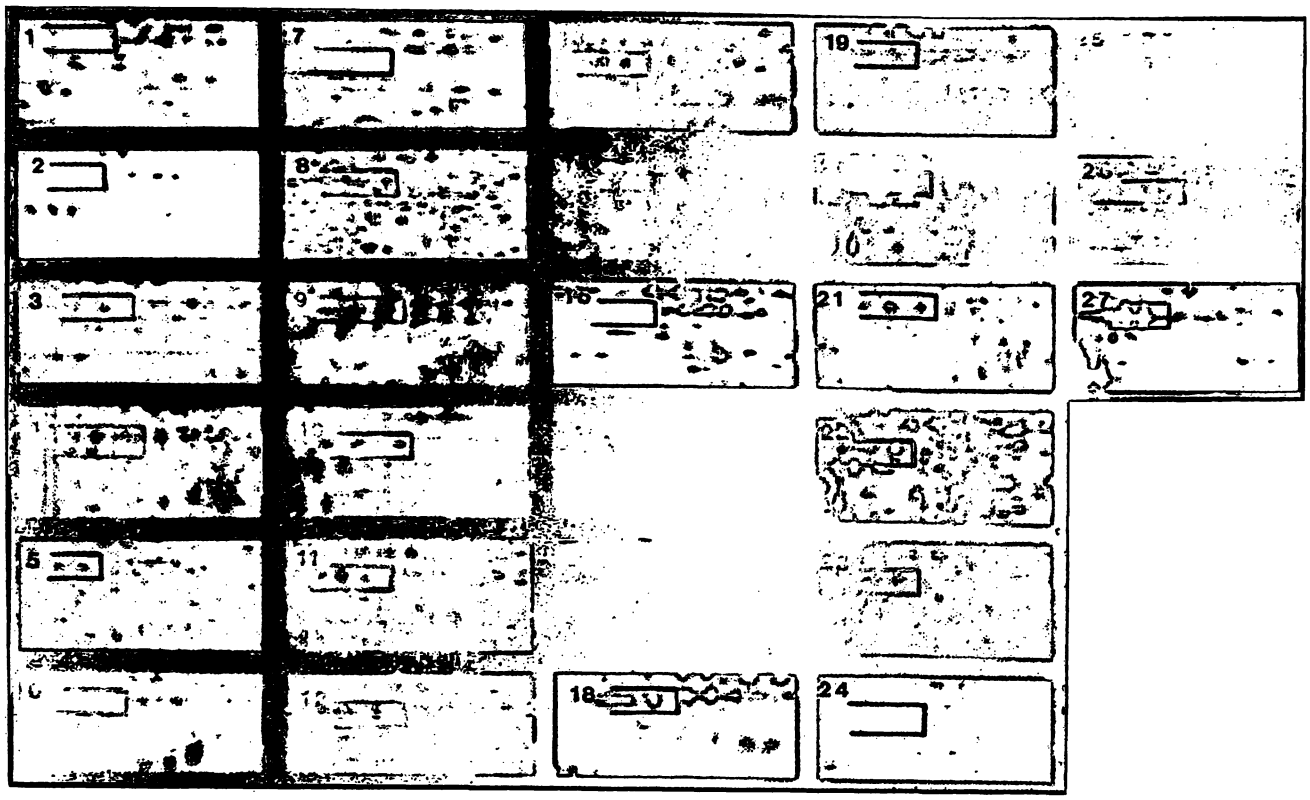

Fig. 6. 2-DE gels area of spot B as marked in figure 3 from the following tissues:

1	buffy coat,	15	liposarcoma,
2	liver,	16	adenocarcinoma of the ovary,
3	skin squamous epithelium,	17	adenocarcinoma of the stomach,
4	mucosa from ileum,	18	adenocarcinoma of the colon,
5	smooth muscle from ileum,	19	renal cell carcinoma,
6 and 7	normal lung,	20	lymph node metastasis from thyroid carcinoma, and cell lines
8 and 9	adenocarcinoma of the lung,		
10 and 11	squamous cell carcinoma of the lung,	21	A-427,
12	leiomyosarcoma in the lung,	22	CALU 3,
13	metastasis of synovial sarcoma in the lung,	23	SK-MES-1,
14	metastasis of epitheloid sarcoma in the lung,	24	SW-480.

tein mixture on the basis of molecular weight and/or isoelectric points is of little value, since these are the separation principles of the 2-DE technique.

In this study our objective was to identify tumour cell-specific proteins and therefore we tried to isolate cells from solid tumours and concentrate them as much as possible. Previous studies analysing solid tumours used entire tissue which contains, beside malignant and normal cells, interstitial substances, plasma proteins, and blood cells which might mask the presence of cell specific proteins or peptides in the 2-DE gels (10–13). Necrosis and proteolysis, which occur to a large extent in neoplastic tissues because of their high metabolic activity, cannot be controlled in whole tissue. Proteins may therefore be cleaved into subunits which appear in different locations on the 2-DE gel or do not show up at all. The increased amounts of low molecular weight proteins on 2-DE gels of entire tumour tissue demonstrate that proteolysis has occurred (fig. 2).

Comparison of normal and malignant tissues

The differences between the 2-DE gels of proteins from malignant lung tumour tissue and adjacent normal lung tissue with no histological evidence of malignant transformation are many and variable, except for the three proteins A, B, and C. Most of the differences are probably inherent in the different cell populations. Normal lung tissue contains brush border cells, exocrine cells, endothelial, epithelial, and mesenchymal cells are present, while each malignant tumour tissue usually consists of one proliferative cell type. We selected and analysed only homogenous and well defined tumour tissues in our study.

The similarity of 2-DE gel patterns of original tumours and their metastases indicate the origin of the metastatic malignant tissue cells from the primary tumour, because neoplastic cells develop from only one malignantly transformed type of cell that reproduces itself as identical malignant cells with similar histological and biochemical features.

Tab. 2. Occurrence of spot A, B, and C in 2-DE pattern of different tissue preparations.

Quantity		Spot A	Spot B	Spot C
	Normal tissue			
16	Lung	6	2	2
2	Skin	2	0	0
2	Mucosa ileum	2	0	2
1	Muscularis ileum	0	1	0
2	Mucosa colon	2	1	2
2	Muscularis colon	1	2	1
1	Parotid gland	1	0	1
2	Liver	0	1	1
1	Testis	0	1	0
2	Buffy coat	0	0	0
31	Total	14 (45%)	8 (26%)	9 (29%)
	Carcinomas including metastases			
15	Adenocarcinoma of lung	15	10	14
12	Squamous cell carcinoma of lung	12	6	12
2	Large cell carcinoma of lung	2	0	2
2	Adenocarcinoma of stomach	2	1	2
1	Hepatocellular carcinoma	0	0	1
3	Renal cell carcinoma	1	1	3
9	Adenocarcinoma of breast	9	1	9
4	Adenocarcinoma of colon	3	1	4
2	Adenocarcinoma of ovary	2	2	2
2	Transitional cell carcinoma of bladder	2	0	2
52	Total	48 (92%)	22 (42%)	51 (98%)
	Sarcomas including metastases			
1	Leiomyosarcoma	0	1	1
2	Synovial sarcoma	0	2	1
2	Lipoid sarcoma	0	2	1
1	Epithelioid sarcoma	1	1	1
6	Total	1 (17%)	6 (100%)	4 (67%)
	Other malignancies			
2	Melanoma	2 (100%)	0 (0%)	2 (100%)
1	Dysgerminoma	1 (100%)	0 (0%)	0 (0%)
2	Lymphoma	2 (100%)	1 (50%)	1 (50%)
	Tissue cultures			
1	CALU-3	1	1	1
1	A-427	1	1	1
1	SK-MES-1	1	1	1
1	A-1663	1	0	1
1	SW-480	1	1	1
1	MEWO	1	0	1
6	Total	6 (100%)	4 (67%)	6 (100%)

2-DE gel patterns of squamous cell carcinoma and adenocarcinoma of the lung did not show any obvious differences which might be useful for subtyping with this technique. Comparison of 2-DE patterns of lung tumours with normal lung tissue showed many variations, but only three spots were consistently present in lung tumours. To investigate if these proteins are lung tumour cell-specific or whether they

also occur in other cells, we looked at several normal tissues: buffy coat for contaminating blood cells, mucosa of the intestine and parotid gland for exocrine cells, muscularis of the intestine for smooth muscle and mesenchymal cells, skin for epithelial cells, testis for germinal cells and liver cells.

We also examined different neoplastic tissues, with different cell types and from different locations to investigate whether some proteins are only present in malignant lung tumours or can also be found in other neoplastic tissues.

Cell cultures are composed of only a single cell type and might exhibit considerable heterogeneity when compared with the original tumour cell (21). In vitro conditions might alter the protein composition of these cells, but some characteristic proteins conceivably could be found in higher concentrations than in resected tissue, which makes them easier to detect by 2-DE. We found tissue cultures to be helpful in identifying tumour-related spots. Additionally tissue cultures are an unlimited source of material for further investigations and have already been used for the production of tumour-associated markers and antibodies (22–27).

Spot A seems to be connected with epithelial tissue and its transformation to a carcinoma. One preparation of the muscularis of the colon showed a small amount of protein A which we suspect to be due to contamination from the mucosal layer. In some preparations of lung, spot A was present which reflects the different cell types of epitheloid and non-epitheloid origin in the lung. None of the sarcomas originating from mesenchymal tissue contain spot A, except for one epitheloid sarcoma.

Spot B seems to be associated with mesenchymal tissue, since its presence is augmented in sarcomas and it occurs in certain normal tissue, but it is also present in some carcinomas.

Spot C seems to be the least specific for any tissue or malignancy of the three described proteins, but it was present in all carcinomas of the lung and in some sarcomas.

This study indicates the presence of specific proteins related to different tissues, but they are also present in their malignant transformations in carcinomas and sarcomas. These proteins are not specific for the type or location of malignant tumours; they are rather common. They allow some differentiation between carcinoma and sarcoma. Spot B occurs in carcinomas and sarcomas whereas spot A is restricted to carcinomas and not to sarcomas. Spot A and B are also present in gastrointestinal tissues. Spot A seems to be a marker protein for epithelial tissue including exocrine organs.

The three specific spots found on the 2-DE have been characterized according to their isoelectric range and molecular weight under denaturing conditions in our 2-DE gel, but they may behave differently in other systems. For this reason it was not possible to compare our results with tumour-associated proteins found by other groups (22–31), even by comparing 2-DE patterns (10–12, 19, 20, 30). Most proteins described in the literature as tumour-associated have an isoelectric point in the acidic range (10, 12, 20, 23, 28, 30) which was also found in our study. Further characterization of these proteins and production of antibodies against these spots may be an additional tool for diagnosis of solid malignant tumours. Cell lines are useful for isolation of these spots, so that these proteins can be further characterized and antibodies can be produced for specific assays (32).

By isolation of neoplastic cells from solid tumours we enhanced the resolution of tumour-associated proteins. Further concentration of the investigated material e. g. isolated membranes or cell skeleton, might provide additional information about tumour-associated proteins on 2-DE gels.

Acknowledgement

This study was made possible by the Max Kade Foundation which supported the leave of Dr. *A. Thomas Endler* from the University of Vienna, Austria. The project was started at the Mayo Clinic and terminated at the University of Pennsylvania. We are grateful to both institutions for financal support, and to Electro-Nucleonics, Inc. for additional support.

References

1. Gold, P. & Shuster , J. (1980) Cancer *40*, 2973–2976.
2. Foley, E. J. (1953) Cancer Res. *13*, 835–837.
3. O'Farrell, P. H. (1975) J. Biol. Chem. *250*, 4007–4021.
4. Anderson, N. G. & Anderson, N. L. (1982) Clin. Chem. *28*, 739–748.
5. Klose, J. & Zeindl, E. (1984) Clin. Chem. *30*, 2014–2020.
6. Goldman, D. & Merril, C. R. (1984) Ann. N. Y. Acad. Sci. *428*, 186–200.
7. Taggart, R. T. & Francke, U. (1982) Cytogenet. Cell. Genet. *32*, 99–110.
8. Celis, E. J., Fey, S. J., Larsen, P. M. & Celis, A. (1984) Proc. Natl. Acad. Sci. U. S. A. *81*, 3128–3132.
9. Leads from the MMWR. (1983) J. Am. Med. Assoc. *252*, 2805–2811.
10. Tracy, R. P., Wold, L. E., Currie, R. M. & Young, D. S. (1982) Clin. Chem. *28*, 915–919.
11. Jellum, E., Thorsrud, A. K., Vatn, M. H., Grimstad, I. A., Brennhovd, I., Tveit, K. M. & Phil, A. (1984) Ann. N. Y. Acad. Sci. *428*, 173–185.

12. Anderson, K. M., Baranowski, J., Olson, L. & Economou, S. G. (1983) J. Surg. Oncol. *24*, 184–191.
13. Stastny, J., Prasad, R. & Fosslien, E. (1984) Clin. Chem. *30*, 1914–1918.
14. Anderson, N. G. & Anderson, N. L. (1977) Proc. Natl. Acad. Sci. U.S.A. *74*, 5421–5425.
15. Tracy, R. P. & Young, D. S. (1984) Clinical application of two-dimensional gel electrophoresis. In: Two-dimensional gel electrophoresis of proteins. (Celis, J. E., and Bravo, R., eds.) Academic Press, New York, NY, pp. 193–240.
16. Oakley, B. R., Kisch, D. R. & Morris, M. R. (1980) Anal. Biochem. *105*, 361–363.
17. Lindena, J., Sommerfeld, U., Höpfel, C., Wolkersdorfer, R. & Trautschold, I. (1983) Enzyme *29*, 100–108.
18. Bravo, R. & Celis, J. E. (1984) Catalog of HeLa cell proteins. In: Two-dimensional gel electrophoresis of proteins. (Celis, J. E., and Bravo, R., eds.) Academic Press, New York, NY, pp. 445–476.
19. Bernal, S. D., Baylin, S. B., Sharper, J. H., Gazadar, A. F. & Chen, L. B. (1983) Cancer Res. *43*, 1798–1808.
20. Ogata, S. I., Ueda, R. & Lloyd, K. O. (1981) Proc. Natl. Acad. Sci. U.S.A. *78*, 770–774.
21. Poste, G. & Fidler, I. J. (1980) Nature *230*, 139–146.
22. Kempner, D. H., Jay, M. R. & Stevens, R. H. (1979) J. Nat. Cancer Inst. *63*, 1121–1129.
23. Velteri, R. W., Maxim, P. E. & Boehlecke, J. M. (1980) Br. J. Cancer *14*, 705–715.
24. Yamada, T., Iwa, T., Kurata, Y. & Okada, S. (1980) Europ. J. Cancer *16*, 781–792.
25. Brown, D. T. & Moor, M. (1982) Br. J. Cancer *46*, 794–801.
26. Brenner, B. G., Jothy, S., Shuster, J. & Fuks, A. (1982) Cancer Res. *42*, 3187–3192.
27. Bernal, S. D. & Spreak, J. A. (1984) Cancer Res. *44*, 265–270.
28. Braatz, J. A., Scharfe, T. R., Princler, G. L. & McIntire, K. R. (1982) Cancer Res. *42*, 849–855.
29. Kasai, M., Saxton, R. E., Holmes, E. C., Burk, M. W. & Morton, D. L. (1981) J. Surg. Res. *30*, 403–408.
30. Pak, K. Y., Blaszcyk, M., Steplewski, Z. & Koprowski, H. (1983) Molec. Immunol. *20*, 1369–1377.
31. Varki, N. M., Reisfeld, R. A. & Walker, L. E. (1984) Cancer Res. *44*, 681–687.
32. Young, D. S. & Tracy, R. P. (1983) Electrophoresis *4*, 117–121.

Dr. A. T. Endler
Institut für Klinische Chemie
und Laboratoriumsdiagnostik
Universität Wien
Lazarettgasse 14
A-1090 Wien